

CLAIMS

Sub
D12

1. A method of screening and recovering a regulatory DNA capable of inducing metastasis comprising the steps of:

i. transferring tagged fragments of a human DNA from malignant, metastatic cancer cells into a cell line that produces only benign, non-metastasizing tumours when injected into a syngeneic animal;

ii. injecting the transformed cells into the syngeneic animal;

iii. selecting those animals in which metastasizing tumours have been identified; and

iv. recovering the regulatory DNA capable of inducing metastasis therefrom.

2. A method as claimed in claim 1 in which the fragments of human DNA transferred in step 1 are from 0.1 to 50 kilo base pairs in length.

3. A method as claimed in claim 2 in which the fragments of human DNA transferred in step (i) are less than 1.6 kilo base pairs in length.

Sub
D1
a

4. A method as claimed in claim 1, 2 or 3 in which the cell line that produces only benign non-metastasizing tumours is a rat mammary epithelial cell line.

5. A method as claimed in claim 4 wherein the rat mammary epithelial cell line is a Rama 37 cell line.

6. A method as claimed in claim 5 wherein the tag is an oligonucleotide sequence:

Primer

Sub
D15

5' AATCCAAGCTTGCGGCCGATCAGGCCGAATATGCGGCCGCATTAT-3'
AGGTTTCGAACGCCGGCTAGTCCGGCTTATACGCCGGCGTAATATCGA

HindIII

SfiI

NorI

Defective
HindIII

AMENDED SHEET

7. A regulatory DNA capable of inducing metastasis consisting essentially of a human DNA fragment of less than 1.6 kilobase pair in length obtained from a malignant, metastasis cancer cell.

8. DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 1:

C2

CTTCCTTGGT	GCTCTATGTC	TTGCCTCTCC	CCTTCTCCAG	TCCCATTAAG	CCATAACCAT
CTTGACAGAC	TCTGGGACAG	TCCCCTCTGC	TCTCCTGTTG	GCGCCTGAGT	CCCTTTTTTGC
CTGAGGACCC	TTACCGTAGC	CTCCCATCTG	GATGACCTAG	TAGAAGACGT	GGGAAGTTGT
CACACTCAGG	TAACTGAGCA	GAGCTCAGAG	ATTTAAAGTG	AGTCTGGGGA	GCCTCGAGGA
TTGATCTGCT	GCCTTAAAAA	GCCAATTGGA	TGACTAACCC	AGACTATTGT	CACTTTAGGT
GGGAAGTCAC	TAGCATATCT	GATGGGTCAC	ATCTGAGAAA	GCTTTCTAGC	AGTGGTGGCC
TTGTGTGAGC	AGCATGGCGT	GTATCATGGT	GTCCAGCATA	CTCAGGCTGC	TTGCAACACT
CGAGGCTCTT	CTTCAGTATT	AGGGGAACCA	CTGGTGTTS	AACATGGTCC	AAGAATACAG
TCATGTGAGG	AGAATCCCAA	TGCGTCAGGA	GAACACGAGA	GTCTGTGACC	TCCATTCTTC
AAGATACAGA	AFTATTCTTG	GACTGTGTTT	TCATGCTCCT	TGTGGATGGG	AGTGAGTTTA
CTTCAGGTTA	ATCAGCATTG	CTTACTGTTG	GTATTCAAGT	AAATGCTTAA	ATTATCCTGG
ATATACCTCT	GTGGGAAGCA	GGTTTTTGAT	ACATGCAGCT	TGTCTTGTG	ATTGATACTG
CTTGAACTCA	AGAGAACTTT	GCTCATGTGA	TCTTTCTTAA	CCGATGGAGT	AGAAACTGTC
TGATGCTCTC	AATAAAGTTG	GCTCTTGCAC	GAGACGTTAG	TCTGTCTGT	TTATCTGCTC
CATTCTTCCG	CTCCCACGGC	CTCTACAGCA	CTAAACCCAC	CACCGATAGA	CTCAGTCTTT
CACCTGACAA	CATCACCAGA	GGCTCTTAAC	TGAGATTATA	AACTGTTACT	AGATGATGGG
TGGAATCGCT	CCCCAGAAAC	ATAAACATTT	ACTTGGAGAA	CTCAAGACCC	CTTTGTAGAC
ATTAATCCCA	TGGT				

9. DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 2:

C5

ATTGCTGTGA	GCCTATTAGC	GACATTTGGT	GACGCCCCCTT	TTAAGGGGGT	AGATACAAAG
AATGGGTTGA	AATTCTGTGC	CACAAACGCT	CTCCATGTTT	TCACAATTAC	ACTTGCAACC
TGTGGTCAGC	AGCCAGAATT	TAGGGATGTG	ATGGGACAGG	GTCCGGGAAA	GAAGGAGAG
GCTAAAGGAA	AGACAGCAGC	TTAAAGTCCA	AACAGCTCCA	GGAGACTATC	TGTAGAAATA
ACATCAGACC	ATGAGGAGAA	TTGATATCAT	TGTTTTTCAA	TGGGTATCGC	CAAGGGAACT
TTCCATCTGA	TTAAAAATTA	TTACTGCTGG	CACTAAATCC	AATTGGAAAT	GCCCCACACA
ATTTATCTTC	CACTTCATGC	TGCTACCATA	TGCTTGACGT	GGCGGAGCAG	AAGCATTCCC
TCCCGTTCTG	ATAAATAGTA	CTTTGTAAAT	ATTTGGAGAC	GGGAGCTCTG	GTGACAGGGA
ACACGTACAA	ACCGGCCCTGT	TTATCATGTT	CCCGATAGAG	GCCCTCTTTG	ACGTACAGGA
CCCCAAAACA	GTCAGGATGC	TGTGAATTTT	CTTCCATGAA	GCCTTGTTCA	CAATTAGCAA
CCATTGGAGG	AAGCAGGCTG	CACTGTCTAC	CACAAAGTGGC	ACTTTCCAAA	GAGCACACAT
ATATTGGAGC	AAGACATTTT	GCTGGCTGAC	TGGTGCTGTG	TAAGCTGATA	AACTGCTATA
TTTATTTAAC	TGGCTTTTCT	TTGAACACCC	CACTCAAGGA	AAAAAAACA	CACTTAGGGT
GACATTATTT	GGAGATGAAG	TCTTTATAGA	GATGCTTAA	TTTAAACGAG	ACTTTTAAAG
CCGGCTCTAT	TCCATTTAAT	GAATGGTGTG	CCTACAAAGG	AAGAACTGG	GACAGAGGTA
TGTACACTTG	TGTGTGTGTG	AGAGACAACG	TGAGGAGCTG	AAGAGGAGCA	CGTACAAATG
AGAGAAAGGC	TGACCCCTTAT	TCACACTGAG	CAAACCAAGT	ATGTGTGGGT	CGATAGATGA
GAGTATCCCC	CAAGACTCAC	ACATTCCGAAC	GCTTGGTC		

10. ✓ DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 3:

C6

AGGACCAGAG	TTCACATCCC	ATCAAATGGC	CCAGAAGGTT	TTAATGCTGT	CTTTTGGCCC
AGGGGCGAAC	TGCACACACA	TGTGCACATA	CACTTACAGA	GACACACATT	CAGCAGCATA
AGAACACAAT	CACAAATAAA	AAAAATCTTG	AAAAATTTTA	AGCTAAAATT	GTTAAGAAAT
AACATATATA	CAATTTTTTCT	TTATTTTTTTT	AAAGATTTAT	TTATTTAATG	TATATGAGTA
CACTGCCTCT	CCCTCCAGAC	ATAGCAGTAC	AGGGCATCGG	ATCCCATTAC	AGATGGTTGT
GAGCCACCAT	GTGGTTTCAC	AGATGGTTGT	GAGCCACCAT	GTGGTTTCAG	GAATTGAACT
CAGGACCTTT	GGAAGAGCAG	TCAGTGCTCT	TAACCTCTAA	GCCATCTCTC	CTGACCCTTA
TATACAATTT	TAATGCTACG	TRACACACAAC	TTCTCTTTCC	TTTAATGGTT	GAGATTTTGT
TCTGGGAAG	TAAGATAAAA	GGAGGGAAG	AACATTGCTT	TCACATTGCA	CCAGTGGGAA
CAGCGTGTTT	AAAGTAGGAA	TGCCATGAAA	TGACTGGCCT	GCCTTCTCAT	TACTGTTCCCT
CCCACCTCCTC	CTTTTAACTG	GAGCTCCTTT	ATCTAATTTA	TTAGTTTGAC	GATACCCAGG
GTTTTCTTCT	GTTTTGATCT	TTTTAAGACA	GAGACTCACC	ATATAGCCCT	GGCTGGCCTG
AAGCTCACTA	TGTAGACCAG	TCTGGCCTTG	AACCTCAAAGG	AGATCTATCT	GCTTCCTAGT
GCTGGGATTA	AAGGCTTGTTG	CTACCAAGTC	TGGTCTGAGG	CTTTGGAGCA	GCCTCGGTTT
TGGCCTTCTT	TAAGGATCTC	TAAGCTAGCA	GTAAGTAGCC	TAGCCATGCT	GTTGTAGGAA
GTTGTTTCGT	CATCCTGGCT	CCAGCACAAA	GGCAGTCACT	AAACGTCCGC	CTCATTTCAT
CAGAGCTGAA	TGCAAATTCC	TTGTGCTCTT	CCTGTGTCCT	CCTGGAAC	

11. DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 4:

C9

AGTTGGGGAC	ACAGCTTGCT	TGATTAAGAT	GTTTCTTGCG	AAAAGGAGTT	AAGCCTAATG
ATTTCCRAATG	GAAAGGACTG	CTAATTGGGG	AGGCAATGTT	GCTTAAATTGG	GACACCTGCG
GGTAATTAAA	AGCTCTCTCC	CAGTGGCCTT	TCCTGATTTT	GGCTCTGGGA	GGCGAAGGCA
TTGAGAGGGA	TGCAGGCATT	CTAAGGGCTG	GTTCTTGTTT	TCTCCCTTCC	CCTCTGTCCA
AACTCAGTGA	GGTATCCCTG	TCTGTGCTGT	CCTTAGAGTG	CCGTCTGAG	GCCTTGGTGA
GTTAAGGTCT	CTGGATCTGA	GCTGCCTCAG	GGAACGCAAT	GAGCTCATTG	GAAAGGGGAG
AACCAGGCAA	AGGTGTTGGC	TGTGACCTCA	GAATTCTGAG	GGGCAAAGGT	TCAAGGCTAA
CTCTCATTAT	AGAGCAAGTT	TGAGACTGGC	CTGGGAACAA	AAATATAAAG	TGAGTGAGGT
CATATGACAG	CACCTGAGGA	GTCCTGTCCC	TAGAGATCAT	AAGGACCTGG	CTGCTGGGGA
CTTGTTGCCAG	ATGGCACTTT	GTGTCGAGAG	AGGGGACCTG	CCCCAGCATG	GGAGGCCCTG
GAAGATCCTC	TGGATTAACT	GTGAACACTG	ATTGCTGCTT	TATACCTGGA	GTTGTGCTGT
TATCTGGTAC	ACATCTGCTG	GGTGAATGAG	TTCATGGGCT	TTATTTTCAGT	GAGGTATTTA
CCTGAGGAGA	AAGAAGGACT	GGTGCCACAA	AGCACAGCTT	TTAAATCTGT	GGGTTGTGAC
CCATTATGGA	CTATCATAAC	TGAGTGACAG	TATCAAGAAT	ACTTTAGCAG	GTGGTAAAAA
GATTTTTTCAA	TGCGCAACGA	CCAAAACTGA	ACTCAAAAAAT	CAAGCATGGC	ATGGATCCTG
GGTGCTCCTG	GAAGCACTTG	CCTTTTACTGC	ATTGTGCGAC	TTGACGGTAG	CCTTGTTTCT
GAATGCACAA	CACGTGGGCT	TTGGGCTGCA	CAGGCCACCA	CGCCGTGCCT	GAAACACCTC
AGCTCAGGTT	TGTGGCTATG	TCCTATGACT	TGGACTTACT	TTTATTGCAC	ATATAAATAT
TTTCCTGC					

12 ✓ DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 5:

C12

```
GAGGGGGTGG TGGCACAGTT ATGTTTTTGT AGGAAGGGTT CCATGAACCT CAGCAGAGCT
CGGGTTAGAA ATTTAAAGC CCTGAGGGGA ATTTTTTTTTT TAAATCGCTA TGAATCTGAC
ATGAGAAAAA CAGATCAGAA ACGTTCTTGT GCTTCAGAAA AGGACAAGTG TGTGACCTAA
CAGACTGCAC ACTGGTGTTC GAGGCACATC TGGATCACAG GAGCGTCAGA TAATGTCCCC
AAAGGTAAAT GCATTTGCTT GCACAGTACC GAGTGTGGTG GGGGGTGCCT ACAGCCCAGC
GTTTCTCAAC CTTCTGTATG CTTGACCCCT TTAATACAGT GCCTCATGCT CTGGTGACCT
CCCCAACCTT AAAATTATTT TTGTTGCTGT TCATAACTGT GATTTTGATA CTGTTATGAA
TTGTAATATA AATAATTTTG AAGAAAGAGG TTTGCCAAGG GTTTGAGAAC TGCTGTTCTA
GCCCCACGTG GATGGTTTTT CGTCATTTGG GGTTTTTTATG AGGCAGAGTC TTATGTAGCC
CAGGCTAGCA GCCTAGAATG TGCTACTTAG CTGAGGAATA ACCTTGGAAC TTCTGAGGAC
TGGAGAGACT GGCTTAGTCC TCAAGAACT GGAATATAGCT GGAGTTTGGC TACTTGTGGG
TTCCTTTTTT TTTCAACCTT TTCTACTCTT TTTCCACCCT GTCGGCCCCC TAACACTAAA
TAAGAAAAGAG AAAGGGGAGC ATAGAGGGGA AAAGAAAACC CTGAATAACG TCAGTAGTTG
GCAAGGGGGG GTGACATATG TTGTCATTAG ACCACATCCT GGTGATTAAG GGGAGTCAAG
TTCCTTGGGG CAGTTTGTAT CTTTCGTGTA ACGATATCTA ATTTCTTCTC CCGTTGCTT
CGTCTTTGTG AACRACGACT TGATAACCCA CAATGGACCA TCAACCAACC AACCAACCAT
```

13 ✓ DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 6:

C20

```
TTGTCTCTGG TGTTACTTGT TTTCCCATTT CTGACAGTGG TTTGACCTT CTATACGCCT
GTGTGTCAAG AGTGCTGTAG ACCTATTTTC CTGTTTTCTT TCAGCCAGTT ACAGGAACAG
AGTGTCTTAC TGTCAGATGT GTAGCTGTTC CTGTCCACTG ACTTTCAAGC TGTCTCTGTG
TGCAGGAACC AGAAGGGCCT GTCCCTACTT CTACTGGGCC CCTACGCACA GGGGGCCTAG
ATGGTGCTAG GTGTTTTTCT CTAGAGCCTG AAATGTGGGC AGAGAGTAGT CTCCTCTGGT
TTCCTAGGTA TGTCTTCCCC TCTGAAGGTC TAGCTCTCCC TTCCATGGGA TATGGGTGCA
GGGAGCTGTT TGACCAGGTC CTCTCAAATC CGGGTGCAGT CTGGACCGCA GGCTCCTGTA
GCTTGCCCTGC TGCAATCTTC CCGCACCCAG AGGCACCCAA GTTTCCCTCTT GGGCCAGGA
TGTGGGCAAA GGTGGGCAGA AGTGGCAATC TCTCCTGCCC TAGCGTCTCA GGATTGCCCT
CACTTCTGGG CAATCCGCTC TCTCTTCCAC AGGGTTTGGG AGCAGGGAGC TGTGGGCCGG
TATCAGGCAA AGGTTTGAGG CAACCAGTTA GAAACTGGAA GTGTCAAGTC CCAGAGGAAT
TTTGCCCTTTG TGTGTCTCTA GTCCACCAGG CAGGTCACTT GGAGCAGAAA AATTGGTTTTT
CCCCTCGGTC TCAGGCCTGA AGETGCACCT CAGGGTTGGC TTTCAGCTGT ACCTGTGGAA
AGTATCGTTT TAAAAATCTA AGATAGCTAT CATGCAGCAA GGCTTGTGTA AAATGTCTAT
TTGGTTCCCT TATGACTTAC TTTTGCTGTA CTGAGGATCA AACCTAGGGT CTCAAGCAGT
CATCACAATT CTCTGTCACT GATCCAGCTC CATTTCTATT TTCTTTTGTG CCGCGCGATC
TCTCGCCAGC AAGAAAACAC GCTAGGGACA TACGAATCCT TGCTGCAGCC AAAACTTTTA
TTGAATCTTA AGGAGAAGCC CGCGCACCGG ACTGGCGCGG TTTATATACA CCCTAGCACA
GTGCATCCAC A
```

14. ✓ The use of an osteopontin gene as a metastasis inducing transformant.

Sub 02 15. A probe specific to a regulatory DNA capable of inducing metastasis as claimed in any of claims 7 to 13.

Sub 01 16. A kit for diagnosing the likelihood of a cancer metastasizing comprising a probe as claimed in claim 15 and one or more of a colour indicator, an oligonucleotide primer, materials for gel analysis and materials for DNA transfer or hybridisation.

Sub 03 17. A medicament adapted to target a regulatory DNA capable of inducing metastasis as claimed in any of claims 7 to 13.

add at

insert